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RESEARCH ARTICLE

Encounter rate and behavior of *Alouatta guariba clamitans* in the Ilha Grande State Park, Rio de Janeiro state, Brazil

Atilla C. Ferreguetti[1], Amanda B.M. de Oliveira[2], Bruno C. Pereira[3], Ricardo T. Santori[4], Lena Geise[2], Helena G. Bergallo[1]

[1]*Departamento de Ecologia, Universidade do Estado do Rio de Janeiro. Avenida São Francisco Xavier 524, Maracanã, 20550-013 Rio de Janeiro, RJ, Brazil.*
[2]*Departamento de Zoologia, Universidade do Estado do Rio de Janeiro. Rua São Francisco Xavier 524, 20550-011 Rio de Janeiro, RJ, Brazil.*
[3]*Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis. Avenida Prefeito Mello Reis 1500, Aeroporto, 36033560 Juiz de Fora, MG, Brazil.*
[4]*Programa de Pós-Graduação em Ensino de Ciências, Ambiente e Sociedade, Departamento de Ciências, Faculdade de Formação de Professores, Universidade do Estado do Rio de Janeiro. Rua Dr Francisco Portela 1470, Patronato, 24435-000 São Gonçalo, RJ, Brazil.*
Corresponding author: Atilla C. Ferreguetti (atilla.ferreguetti@gmail.com)

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ABSTRACT. *Alouatta guariba clamitans* Cabrera, 1940 is an endemic species of the Atlantic Forest that occurs from south Bahia, Brazil, extending south to the province of Misiones, Argentina. In Rio de Janeiro state, the species was classified as threatened, indicating that attention is needed for the conservation of this taxon. Additionally, an outbreak of yellow fever spread throughout the southeastern states of Brazil from January 2017 until March 2018 seriously threatening Rio de Janeiro populations of the species. Herein, we aimed to provide the first estimates of *A. g. clamitans* encounter rate, density, and population size in the Ilha Grande State Park (PEIG), which is part of the Atlantic Forest biome of Brazil. Data were collected in two different periods, the first between December 2003 and May 2005, and the second from August 2009 to May 2010, and information on encounter rates and behavior was collected to better understand aspects of species' ecology. The estimated encounter rate in the first period through the distance sampling method was 0.04 ± 0.01 individuals per kilometer. Nine groups were recorded in the second period of the study, with 47 individuals along 3 km. Our estimates of encounter rate, density and population size were low and reinforces the need to initiate species monitoring and assess the impact that yellow fever outbreaks may have on PEIG populations. The results presented here can be a starting point to support future strategic actions for the species, to measure impacts and to the management of the species, and for a conservation program.

KEY WORDS. Distance sampling, linear transect, primates, scan-sampling, Southern brown howler monkey.

INTRODUCTION

Alouatta guariba clamitans Cabrera, 1940 is an endemic primate species of the Atlantic Forest (Kinzey 1982, Horwich 1998, Mendes et al. 2008), occurring in the region of Misiones, Argentina (Di Bitetti et al. 1994), and in Brazil, from the extreme south, in the region of Canto Galo, state of Rio Grande do Sul (Printes et al. 2001) to the mouth of the Jequitinhonha river, southern Bahia state (Rylands et al. 1996). Individuals may be sexually defined by dichromatism in their pelage as the adult male has a reddish color due to the secretion of exocrine glands (Hirano et al. 2003) and the adult females have a darker pelage with the dorsal coat and the pelage of the members varying from brown blackish to reddish brown (Gregorin 2006).

In the Rio de Janeiro state, *A. g. clamitans* occurs throughout the coast (Gregorin 2006) and although it has been found in fragmented areas (Chaves and Bicca-Marques 2013) it has been considered threatened and classified as Vulnerable (Bergallo et al. 2000, Bicca-Marques et al. 2015, ICMBio 2018), pointing to a higher conservationist attention for the taxon (Bergallo et al. 2000). Poaching and habitat destruction are the main factors that negatively affect the populations of *A. g. clamitans*

(Bergallo et al. 2009, Monticelli and Morais 2015). However, the contemporary outbreaks of yellow fever is another factor threatening the populations of *A. g. clamitans* and other South American primates that reinforcing the need for studies on their ecology and behavior (Bicca-Marques 2009). In January 2017, an outbreak of yellow fever began to spread through the southeastern states of Brazil (Fernandes et al. 2017). Yellow fever is a disease caused by a virus of the family Flaviviridae, carried by mosquito vectors *Haemagogus* Linnaeus, 1758 and *Sabethes* Sabethes Robineau-Desvoidy, 1827 (sylvatic cycle) and *Aedes aegypti* Linnaeus, 1762 (urban cycle) (Barrett and Monath 2003). Humans and non-human primate species show different levels of susceptibility to the disease (Kumm and Laemmert 1950, Hervé and Travassos da Rosa 1983, Thoisy et al. 2004). For non-human primates in the Atlantic Forest, yellow fever was diagnosed in 150 of 1000 primate individuals tested (15% occurrence) from January to July 2017 (Fernandes et al. 2017).

Among South American primates, the genus *Alouatta* Lacépède, 1799 (howler monkeys) appears to be the most susceptible (Araújo et al. 2011). For this reason, howler monkeys are considered sentinel species for the early detection of yellow fever epidemics (Araújo et al. 2011).

Information for *A. g. clamitans* on the island named Ilha Grande is restricted to its occurrence and there are no systematized works on the ecology and behavior of the species (Alho et al. 2002, Pereira et al. 2017). Estimates of population size indicators are critical to any effort to conserve endangered species. These estimates allow the assessment of anthropogenic impacts on natural populations, such as habitat loss, identify priority areas for conservation, assess the viability of isolated populations, determine the conservation status of the species, and serve as a basis for other ecological studies as well as important tool for decision makers (Cunha and Loyola 2011, Buckland et al. 2016, IUCN 2018).

In the Ilha Grande State Park (PEIG in Portuguese, Parque Estadual da Ilha Grande), at least 28 individuals of *A. g. clamitans* were found dead in trails in 2017 (personal communication, T. Barradas – Head of Ilha Grande State Park at the time). This number is probably underestimated, because possibly individuals who died inside the forest, away from tracks, were not accounted for. In this context, we aimed to provide the first estimate of the encounter rate and information about the behavior of the species in PEIG.

MATERIAL AND METHODS

We conducted the study in the Ilha Grande State Park (PEIG hereafter), located in the Ilha Grande island, southwestern coast of Rio de Janeiro state, Brazil (Fig. 1). The island is isolated from the mainland for about 2 km, with some mountains reaching 1000 m in altitude (Callado et al. 2009, INEA 2010). PEIG is the second largest insular biological park in Brazil and covers 120 km², over half (62%) of the island, which has 193 km² (INEA 2010). The climate is hot humid tropical without a dry season. Ilha Grande is the top of a submerged mountain and has two dominant types of topography, mountain and coastal plain (INEA 2010). Almost half of the area (47%) is covered by dense, relatively pristine Atlantic rainforest. Disturbed forests, in an advanced successional stage, are the second major habitat type (43%). The remaining areas comprise rocky outcrops with herbaceous vegetation (7%), salt marshes, mangroves and beaches (2%), and human settlements representing 1% of the island (Alho et al. 2002, Oliveira 2002, Callado et al. 2009). Human settlements are mainly concentrated around the northern coastline of Ilha Grande Bay and in Abraão village.

Encounter rate, density and population size

We collected data between December 2003 and May 2005, using 127 samplings, totaling 397.3 km walked in 382 hours of effort. We performed samplings on five existing trails in Ilha Grande to minimize the impact of opening new trails (Fig. 1, Table 1). These five trails covered all types of vegetation found on the Ilha Grande and crossed several streams. Two of these trails are located in the northern part, linking the island's largest settlement, Abraão Village, to the beaches of Palmas (length 2.1 km) and Feiticeira (2.3 km) (T01 and T02, respectively). The other three trails are on the south side of the island, connecting Dois Rios Village to the beaches of Caxadaço (2.7 km) and Parnaióca (6.7 km), and to the place locally known as Jararaca (2.1 km), T03, T04 and T05, respectively. We walked the transects early in the morning (5:30 am) and afternoon (3:30 pm), lasting an average of three hours, and walked on average 25.6 times ranging from 23 to 27 times, with an average speed of 1.1 km/h (± 0.5).

For each observation of *A. g. clamitans*, we recorded the perpendicular distance of the first animal sighted from the transect using a measure tape, the length of the transect walked to that point where the animal was observed, date and time of the observation. Encounter rate, density and population size were estimated using the total number of individuals observed within the PEIG (120.52 km²) using the DISTANCE software version 7 (Buckland et al. 2001). This software uses the perpendicular distances to observed animals to estimate the Effective Strip Width (ESW) in the study area and to model the detection function that best fits the probability of detection of an animal at a given distance (Buckland et al. 2001). The best detection model was selected by the Akaike Information Criterion (AIC, Akaike 1973).

Group composition and behavior

We collected data from August 2009 to May 2010. We used the first 3 km of the T04 transect (Fig. 1) to characterize the following aspects: group characterization and composition, activity and main behaviors. In total, the groups were followed up for 37 days, with 141 hours of field effort.

We performed groups identification according to their gender-age composition, based on Mendes's (1989) adapted classification, as well as on the individual characteristics of the

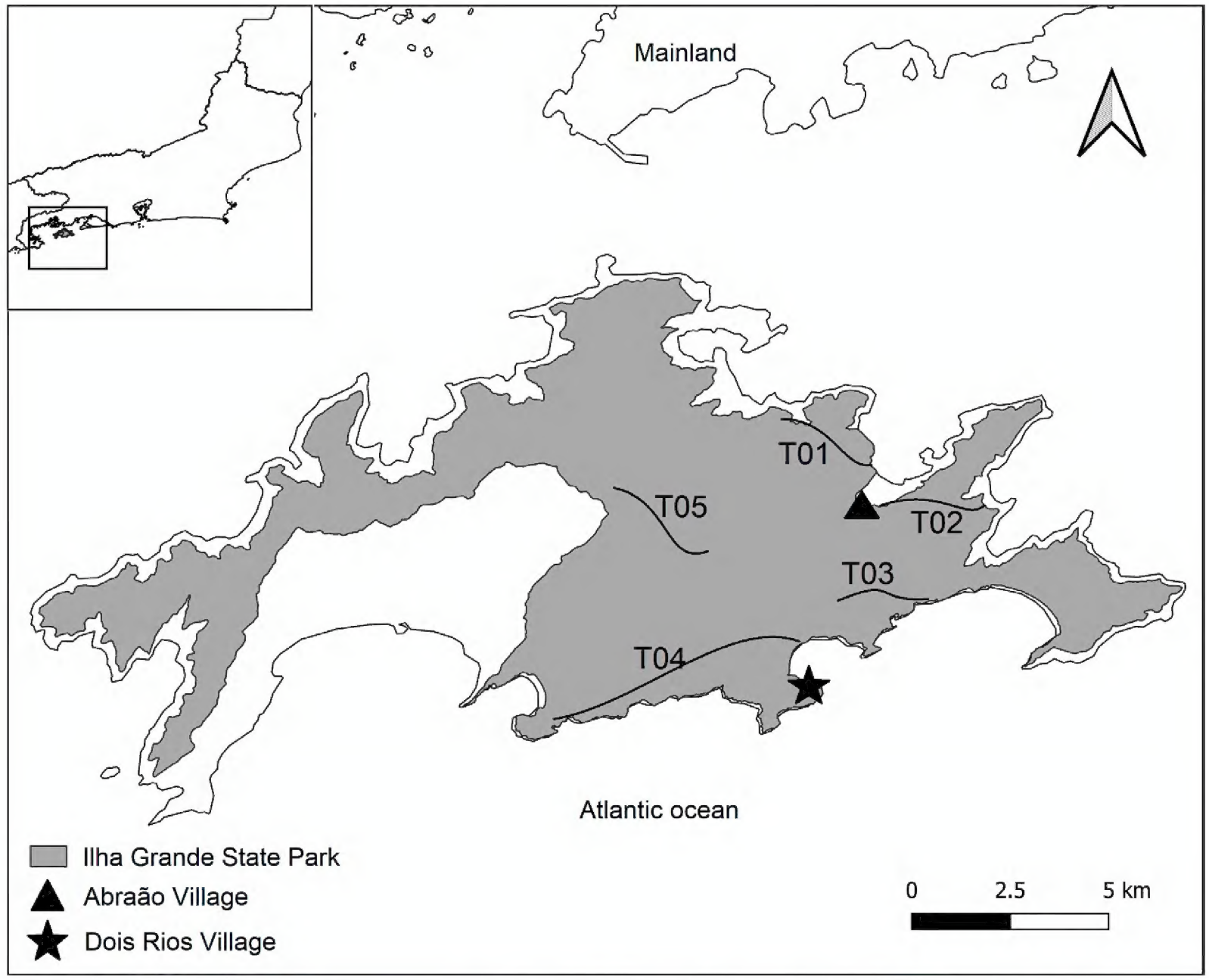

Figure 1. Map of the study area, showing the area covered by the Iha Grande State Park, in Ilha Grande, located in the southwest part of the Rio de Janeiro, Brazil. The black lines indicate the transects.

Table 1. Characteristics of transects (length and coordinates), number of times each transect was walked (N of samples) and total kilometers walked. The coordinates refer to a central point of the tracks. The study was conducted from December 2003 to May 2005, Ilha Grande, Rio de Janeiro, Brazil.

Transect	T01	T02	T03	T04	T05	Total
Coordinates	23°07'37.4"S, 44°10'47.3"W	23°08'43.3"S, 44°08'53.5"W	23°10'07.9"S, 44°10'24.9"W	23°11'26.2"S, 44°13'43.5"W	23°09'25.4"S, 44°14'19.6"W	
Length (km)	2.1	2.3	2.7	6.7	2.1	15.9
N of samples	27	23	27	24	27	128
Total effort walked (km)	56.7	52.9	72.9	160.8	56.7	401
Observed groups	0	0	1	6	9	16

animals, such as coat color, size and scars. For this, we determined that the time of contact with the animals should not be less than 30 minutes and that all individuals detected in the group were described (Mendes 1989). Groups that did not have all the members described, due to poor visualization or withdrawal of individuals during identification, were not included in the results. The observation sessions started at 7:00 am and were finished at 05:00 pm. For the distinction between morning and afternoon shifts, it was considered "morning" from 7:00 am to 12:00 pm, and "afternoon", from 12:01 pm to 5:00 pm.

We performed behavioral records with direct observation and using Bushnell™ 10 × 50 mm binoculars. Observations consisted of records for each group found, characterizing an observation session, which consisted of the first visualization of any group or individual, until it was no longer within the reach of the observer, thus characterizing the end of the session. Therefore, we recorded behaviors of distinct groups that used the area close to the trail walked.

We used the method of scan-sampling (Altmann 1974), where the whole group is quickly examined at regular intervals, generating individual records, with observations every 15 minutes, with 5 minutes for scanning and 10 minutes for interval (Bravo and Sallenave 2003, Ludwig et al. 2008, Prates and Bicca-Marques 2008) to reduce statistical dependence (Setz 1991). The sampled categories of activity were resting, feeding, movement and social behavior (Table 2). We recorded social behaviors and described through the sampling of "all occurrences", which characterize opportunistic observations used to develop ethograms and for observations of rare but important behaviors (Lehner 1996). For the quantification of social behaviors, we grouped the categories in vocalization, grooming, agonistic behavior, marking, playing and sexual behavior.

Table 2. Behavior categories of *Alouatta guariba clamitans* sampled using the scan-sampling method in the Ilha Grande State Park, Rio de Janeiro state, Brazil.

Category	Description
Resting	Individual inactive, standing, sitting, lying, quadruped or hanging by tail
Feeding/Foraging	The act of selecting and picking up the food, chewing it and swallowing it
Movement	Displacement in the same tree or between trees
Social behavior	Behaviors that involve some type of interaction between animals, such as the categories of grooming, playing, agonistic behaviors, sexual behaviors and vocalization

We grouped the data from each scan by the number of individuals in the group who were performing a certain activity at a given time. Then, we analyzed these records obtained by scan-sampling of each behavioral category for the total period of study. For calculating the percentage of each category i, we consider: pi = ni/N × 100, where pi: percentage of category i, ni: the number of records of category i during the period under analysis, and N: the total number of records of all categories during the same period. Where: i = resting, feeding, movement or social behavior.

We performed Spearman correlations between pairs of activity variables to determine possible relationships between activities (Zar 1996). To compare the categories of social behaviors sampled, we used the chi-square test. We performed the analyses in the statistical environment R (R Development Core Team 2010).

RESULTS

Encounter rate, density and population size

We obtained 16 observations of groups of *A. g. clamitans* in the 397.3 km covered. The effective strip width (ESW) was 8.42 ± 2.14 m with records obtained from 0 to 18.5 m from the transect line. The data were best fitted to a half normal curve with cosine adjustment and a correction following the Poisson distribution. The estimated density was 1.09 ± 0.39 groups/km^2 (Confidence Interval – CI 0.7–1.48), with an estimated group size of 4.21 ± 1.76 individuals (CI 2.45–5.97) and total estimated density was 4.58 ± 1.58 individuals/km^2 (CI 3.00–6.16). The estimated population size for the whole PEIG was 505 ± 211 individuals, with a confidence interval of 294 to 716 individuals. However, the coefficient of variation for density, size of groups and population size was 36.17%. In cases where the coefficient of variation was above 20%, which is considered the limit for a reliable estimate we used the encounter rate. The encounter rate in the PEIG was 0.04 ± 0.01 groups per km traveled (CI 0.03–0.05).

Group composition and behavior

We obtained 2,268 individual records in 698 scan-samplings. Using the "all occurrences Method", we obtained 190 records of social behaviors distributed over 31 days. We identified 47 individuals, distributed in nine distinct groups and two solitary male individuals. All nine groups observed were composed of individuals of both sexes. The mean groups size (± standard deviation) was 5 ± 1.7 individuals (ranging from 2 to 7 individuals). Social composition per group was represented by one to two adult males (1.1 ± 0.3 individuals), one to three adult females (1.9 ± 0.6 individuals) and from zero to four immatures of different age (2 ± 1.3 individuals). Only one observed group consisted of two adult males. The groups were composed of 22% of adult males, 38% of adult females, 4% of subadult males, 27% of juveniles, and 9% of infants. Only one group of adults was observed. The sex ratio between males and females was 1: 1.7. Considering infants, juveniles and subadults as immature, the immature-to-female ratio (IFR) was 1.06 and immature-to-adult ratio (IAR) was 0.67.

The most common behavior observed was resting (45.2%), followed by feeding (28%), movement (21.7%) and social behavior, which occurred in a small fraction of the day (5.1%). Significant negative correlations were found between the percentages allocated for resting and feeding (rs = -0.77, p = 0.021, df = 7, n = 9), as well as for the combination between resting and movement (rs = -0.9, p = 0.002, df = 7, n = 9). The correlation analyzes between the other activities did not present statistically significant correlations (Table 3). In total observations, within the sampled period, individuals exhibited resting

Table 3. Spearman Correlation values (upper right) and level of significance (lower left) among the percentages displayed for each activity of *Alouatta guariba clamitans* behavior in Ilha Grande State Park, Rio de Janeiro state, Brazil. Bold values were statistically significant.

Behavior	Resting	Feeding/Foraging	Movement	Social
Resting	–	-0.77	-0.90	-0.37
Feeding/Foraging	**0.021**	–	0.52	0.60
Movement	**0.002**	0.162	–	-0.02
Social	0.336	0.097	0.982	–

behavior predominantly in the range of 8:00 to 10:00 am. The feeding activity was more present in the afternoon shift, with a peak between 02:00 and 05:00 pm. The social behavior was more displayed in the morning (07: 00–08:00 am) and in the middle of the day (11:00 am –12:00 pm) (Fig. 2).

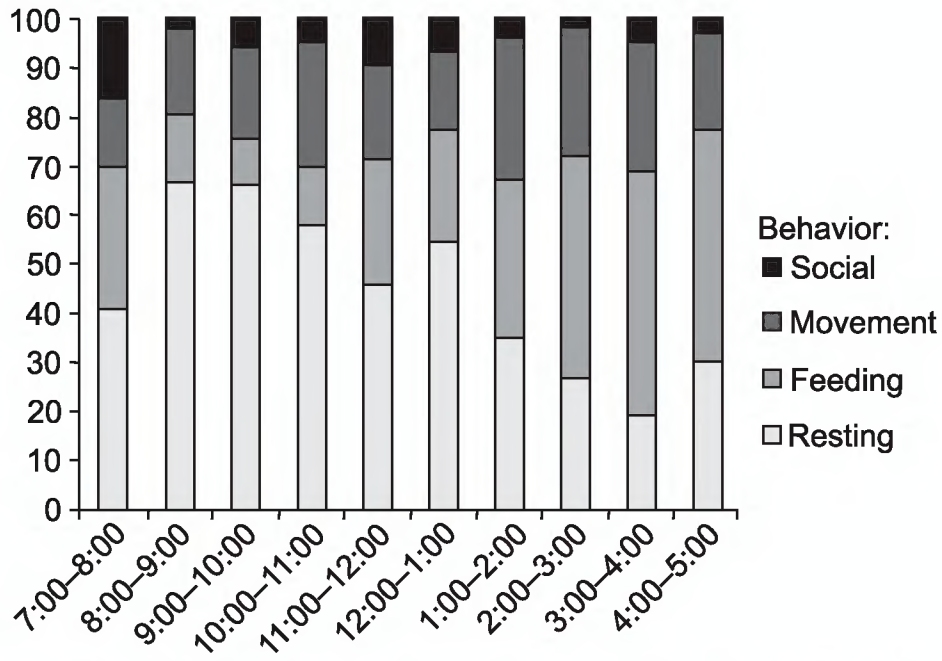

Figure 2. Average observations of *Alouatta guariba clamitans* activity patterns from August 2009 to May 2010 in the Ilha Grande State Park, Rio de Janeiro, Brazil.

Feeding was characterized predominantly by the consumption of leaves (young or mature), besides sprouts, fruits and flowers. We were able to identify in field three plant species used as food resources: flowers and fruits of *Miconia prasina* (Sw.) DC. (Melastomataceae), embaúba leaves (*Cecropia glazioui* Snethl.: Urticaceae) and, most commonly, fig leaves and fruits (*Ficus vermifuga* (Miq.) Miq.: Moraceae).

There was a significant difference in the display of categories of social behavior. The most commom social behavior was vocalization, with 45.8% of the records, followed by grooming (33.7%), agonistic (7.9%), playing (5.8%), marking (4.2%) and sexual behavior (2.6%) (χ^2= 192.10, $p < 0.001$, df = 5).

DISCUSSION

To compare densities among studies, it is very important that the studies be conducted following the same methodologies. Therefore, estimated density of individuals of *A. g. clamitans* for the PEIG using the distance sampling (average of 5 ind./km^2) can be considered low compared to other studies with this taxon (Table 4). Higher values of density in other areas of the Atlantic Forest has been estimated, such as, for example, 81 ind./km^2 in the Cantareira State Park in São Paulo state (Silva-Junior 1981), 15 ind./km^2 (Chiarello 1999) and 60 ind./km^2 (Ferreguetti et al. 2016) in the Vale Natural Reserve, located in the north of Espírito Santo state. In Rio de Janeiro state, Araújo et al. (2008) estimated 44.1 ind./km^2 in the Poço das Antas Biological Reserve and 42.1 ind./km^2 in the União Biological Reserve, both estimates are much higher than the present study estimate. However, the estimated encounter rate fits within the confidence interval of previous studies. Encounter rates of the species is variable in the literature (0.01–2.3 individuals per km walked, Table 4) and here, we estimated 0.4 individuals per km walked. The mean group size was similar in the two study periods using two different methods (4 and 5 individuals, respectively). Previous studies have argued that the size of *A. g. clamitans* groups ranges from 2 (Silva-Junior 1981, Chiarello 1992, Pinto et al. 1993) to 13 individuals (Jardim 2005), and the mean size of 3.7 (Pinto et al. 1993) to 8.23 (Jardim 2005) individuals per group. Therefore, the groups size found in the PEIG fits within the range of these studies.

The occurrence of solitary male individuals in this study is consistent with the predominance of disappearances of young males, subadult and adult observed by Jardim (2005). According to the author, this disappearance is related to the intra-sexual competition and *Alouatta*'s polygynous social system, in which the group is generally composed of one to two adult males and two to three adult females (Silva-Junior 1981, Mendes 1989). The entry into other groups is possibly related to the physical capacity of adult males and the ability to form alliances and coalitions with related individuals to define the hierarchy in the group (Jardim 2005). The formation of most groups of *A. guariba* with one male in its composition was observed by several authors (Table 5). Thus, groups identified in the PEIG followed the trend presented for the species (Table 5).

The ratio between the sex-age classes of the individuals, such as the ratio between males and females, the proportion of IFR and the proportion of IAR are relevant factors to be considered in the composition of the groups, which are used as indicators of population status (Rumiz 1990, Clarke et al. 2002). In fact, adult females generally correspond to the highest proportion of adult individuals (Crockett and Eisenberg 1987, Rudran and Fernandez-Duque 2003, Aguiar et al. 2009). The male/female sex ratios (1:1.7), as well as the calculated values for the IFR (1.1) and IAR (0.7) parameters confirm what has been observed in studies developed in other areas for the genus (Table 5). Lower values of these indices indicate that the population faces difficulties or is declining, while higher values indicate more viable groups or populations (Zucker and Clarke 2003). Although Heltne et al. (1975) have suggested that an IFR below 1.5 is critical for the survival of a population, lower values have been reported for *Alouatta palliata* (Gray, 1849), with no evidence of decline (0.75: Clarke et al. 2002, 0.62: Zucker and Clarke 2003). Therefore, the results may suggest a relative stability between the groups studied in the PEIG at the time.

In general, the activity pattern of the groups observed was similar with most results obtained in other studies for the species and for the genus, because higher frequency activity was resting, followed by feeding activities, movement and social behaviors (Mendes 1989, Chiarello 1992, Oliveira and Ades 1993, Martins 2008). This high inactivity of the howler monkeys can be explained by the low energy value of their folivorous diet and

Table 4. Estimates of population density and encounter rate for *Alouatta guariba clamitans* in different areas of the Atlantic Forest

Locality	Density (ind/km²)	Encounter rate	Study
Ilha Grande State Park, RJ	4.58	0.04	Present study
Vale Natural Reserve, ES	60	–	Ferreguetti et al. (2016)
Ilha do Cardoso State Park, SP	10.6	–	Ingberman et al. (2009)
União Biological Reserve, RJ	44.1	0.07	Araújo et al. (2008)
Poço das Antas Biological Reserve, RJ	42.1	0.11	Araújo et al. (2008)
Viraeiro/Tabatingüera, SP	27.1	0.14	Martins (2005)
Sara, SP	34.6	0.22	Martins (2005)
Água Sumida, SP	10.42	0.01	Martins (2005)
Monal, SP	8.32	0.006	Martins (2005)
Morro do Diabo, SP	15.6	0.07	Cullen et al. (2001)
Tucano Farm, SP	10.9	0.07	Cullen et al. (2001)
Mosquito Farm, SP	36.3	0.07	Cullen et al. (2001)
Caetetus Ecological Station, SP	0.6	0.07	Cullen et al. (2001)
Rio Claro Farm, SP	16.3	0.07	Cullen et al. (2001)
Paranacicaba, SP	0.8	0.02	González-Sólis et al. (2001)
Vale Natural Reserve, ES	15	0.01	Chiarello (1999)
Serra do Brigadeiro, MG	7.5	–	Cosenza and Melo (1998)
Caratinga, MG	149	–	Hirsch (1995)
Rio Doce State Park, MG	49	–	Hirsch (1995)
Augusto Ruschi Biological Reserve, ES	10.1	0.18	Pinto et al. (1993)
Cantareira State Park, SP	81	–	Silva-Junior (1981)

Table 5. Studies developed with *Alouatta guariba* and *A. guariba clamitans* in other localities that evaluated the same behavioral parameters of the present study at Ilha Grande State Park, Rio de Janeiro state, Brazil.

Locality	Groups with one adult male (%)	Sex ratio (M/F)	Immature to female ratio (IFR)	Immature to adult ratio (IAR)	Study
Ilha Grande State Park, RJ	90	1 : 1.7	1.06	0.67	Current study
Cantareira Reserve, SP	64	1 : 1.31			Silva-Junior (1981)
Caratinga Biological Station, MG	84	1 : 1.2	1.3	0.9	Mendes (1989)
Intervales State Park, SP	83	1 : 1.85	1.2	0.8	Steinmetz (2001)
Morro da Extrema, RS	60–90	1 : 2.25			Jardim (2005)
Fragmento Mata do Lami, RS		1 : 3			Jardim (2005)
Parque Estadual de Itapuã, RS		1 : 2.6			Jardim (2005)
Chácara Payquerê do Bugre, PR		1 : 1.57	1.4	0.8	Miranda and Passos (2005)
Parque Estadual da Ilha do Cardoso, SP	80	1 : 1.58			Ingberman et al. (2009)

strategic conservation of energy (Milton 1998). The percentage displayed for resting behavior, when compared to other studies, where ranges from 53.1% (Marcos de Souza Fialho, unpublished data) to 74.1% (Limeira 2000), was lower (45.2%). However. Flávia Koch (unpublished data) found similar movement values to this study in an Atlantic Forest fragment located in the municipality of Barra do Ribeiro, Rio Grande do Sul state, Brazil. One factor that may have influenced this lower proportion of resting behavior was the observation period. We did not conducted sampling in the late afternoon nor early evening, where activities are reduced, allowing for an underestimation of resting behavior. However, Oliveira and Ades (1993) began their observations at 8:00 am and this did not result in a reduction in resting time observed, when compared to other studies. Vocalization was the most frequent social behavior (45.8%), as well as observed by Moro-Rios et al. (2006) (53.4%). In the study by Marcos de Souza Fialho (unpublished data), vocalization and playing were characterized as predominant social behaviors (45.6% and 43.6% in winter and 26.5% and 30.1% in summer, respectively). Oliveira and Ades (1993) and Martins (2008) found a higher percentage of grooming and playing behaviors (1.9% and 1.1%, 1.2% and 1.6%, respectively, relative frequency of records of all activities) than vocalization (0.4% and 0.6%, respectively, of all activities). However, these percentages may present variations according to the composition of the observed groups, such as the presence of immatures, which can increase the playing records. Another factor that may interfere with the number of vocalization records is the distribution and density of the groups present in the study

area, because vocalization can be associated with territoriality behavior in vocal confrontations (Horwich and Gebhard 1983, Bonvicino 1989). On the other hand, some authors affirm that individuals of *A. g. clamitans* are not territorial, but only antagonistic to those that are not part of their groups, and that defend the place where they are (Neville et al. 1988).

Our estimates of the encounter rate, density and population size of *A. g. clamitans* in the PEIG were low and reinforce the need to initiate a monitoring of the species to assess the impact that outbreaks of yellow fever may have on PEIG populations. The PEIG is an important area for the conservation of the species in the Rio de Janeiro state, and it should be noted that some individuals were victims of yellow fever in 2017. The risk of the species being locally extinct may be higher if new outbreaks of yellow fever reach the Ilha Grande, because it is an island population isolated without immigration from mainland individuals. Therefore, the results presented here can be a starting point to support future action plans for the species *A. g. clamitans*. Population estimates and behavioral information presented here could help to understand species relationships within the PEIG and may be important for assist management measures for the conservation of this species.

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